

REVIEW ARTICLE

H₁-antihistamines: inverse agonism, anti-inflammatory actions and cardiac effects

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Summary

This review addresses novel concepts of histamine H₁-receptor function and attempts to relate them to the anti-inflammatory effects of H₁-antihistamines. Furthermore, the molecular mechanisms underlying the cardiotoxic effects of H₁-antihistamines are discussed.

H₁-receptors are G-protein-coupled-receptors (GPCRs), the inactive and active conformations of which coexist in equilibrium. The degree receptor activation in the absence of histamine is its 'constitutive activity'. In this two-state model, histamine acts as an agonist by combining with and stabilizing the activated conformation of the H₁-receptor to shift the equilibrium towards the activated state. Drugs classified previously as antagonists act as either inverse agonists or neutral antagonists. Inverse agonists combine with and stabilize the inactive conformation of the receptor to shift the equilibrium towards the inactive state. Thus, they may down-regulate constitutive receptor activity, even in the absence of histamine. Neutral antagonists combine equally with both conformations of the receptor, do not affect basal receptor activity but do interfere with agonist binding. All H₁-antihistamines examined to date are inverse agonists. As the term 'H₁-receptor antagonists' is obviously erroneous, we suggest that it be replaced by 'H₁-antihistamines'.

The observations that H₁-receptors modulate NF-κB activation and that there are complex interactions between GPCRs, has allowed us to postulate receptor dependent-mechanisms for some anti-inflammatory effects of H₁-antihistamines, e.g. inhibition of ICAM-1 expression and the effects of bradykinin.

Finally, the finding that blockade of HERG1 K⁺ channels is the mechanism by which some H₁-antihistamines may cause cardiac arrhythmias has allowed the development of preclinical tests to predict such activity.

Introduction

The past 5 years have seen tremendous advances in our knowledge about the mechanisms by which H₁-antihistamines produce their effects, both beneficial and unwanted. This review concentrates on recent developments in three areas of antihistamine biology: the molecular mechanisms by which antihistamines interact with histamine receptors; the possible

anti-inflammatory actions of H₁-antihistamines; and the mechanisms, both genetic and pharmacological, by which they produce unwanted effects on the heart.

H₁-antihistamines are inverse agonists rather than receptor antagonists

Histamine receptors are classified into four subtypes: H₁, H₂, H₃ and H₄, all which belong to the superfamily of G-protein-coupled receptors (GPCRs) [1]. The G_{q/11}-coupled H₁-receptor, encoded on chromosome 3, is responsible for many symptoms of allergic disease, including rhinorrhoea, contraction of bronchial and gastrointestinal smooth muscle and many forms of itch. Activation of the H₁-receptor-coupled G_{q/11} stimulates the inositol phospholipid signalling pathways resulting in formation of inositol-1,4,5-triphosphate (InsP₃) and diacylglycerol (DAG) and an increase in intracellular calcium [2]. In addition, the H₁-receptor can activate several other signalling pathways, including phospholipase D and phospholipase A₂ [2]. More recently the H₁ receptor has also been shown to activate the transcription factor NF-κB [3–5]. As both the H₁-receptor and NF-κB are known to be involved in allergic conditions, it is attractive to speculate that in such disorders the coupling of the H₁-receptor to the NF-κB pathway is of physiological importance.

Historically, the potency of H₁-antihistamines has been assessed using a variety of standard pharmacological assays, particularly contraction of guinea-pig ileal or tracheal smooth muscle. In these tissues, the drugs cause a parallel shift in the concentration-response line to histamine. This is consistent with their being competitive antagonists of histamine at its H₁-receptor and lead to the formal classification of the drugs as H₁-receptor antagonists.

Over the past few years, however, advances in molecular biology, particularly in the expression of GPCRs in recombinant cell systems, have altered our understanding of how agents interacting with GPCRs may exert their effects. Classical models of GPCRs require the occupation of receptors by an agonist to initiate activation of signal transduction pathways. However, it has been shown recently that GPCRs can have spontaneous activity which is independent of receptor occupancy by an agonist. This is referred to as constitutive receptor activity [6]. The concept of constitutive activity has led to a reclassification of drugs acting at GPCRs (Fig. 1). Ligands classified previously as antagonists may now be redefined as either inverse agonists, i.e. ligands which are able to reduce constitutive GPCR activity, or neutral antagonists, i.e. ligands which do not affect the basal GPCR activity but do interfere

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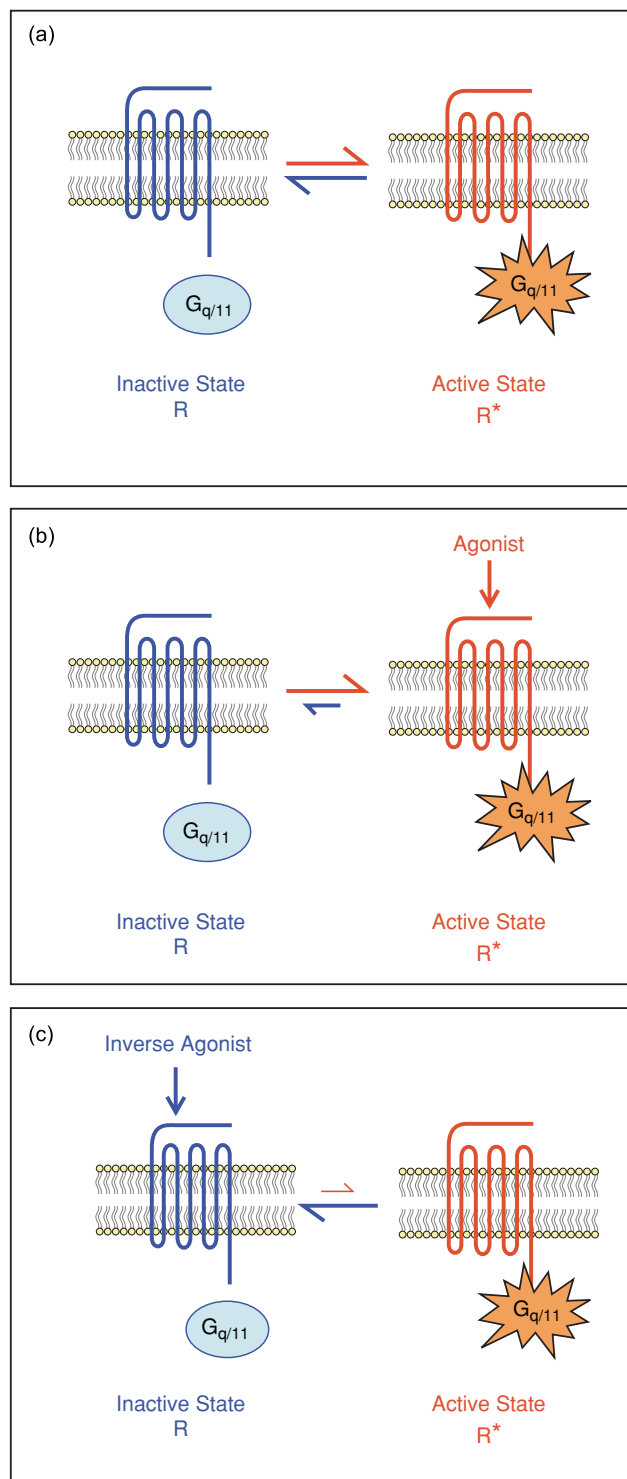


Fig. 1. Simplified two state model of the histamine H₁-receptor. (a) at rest, the inactive state (R) isomerizes with the active state (R*) and vice versa to set up an equilibrium between the two states. (b) an agonist, which has a preferential affinity for the active (R*) state, stabilizes the receptor in this conformation and, consequently, causes a shift in the equilibrium towards the activated (R*) state. (c) an inverse agonist, which has a preferential affinity for the inactive (R) state, stabilizes the receptor in this conformation and, consequently, causes a shift in the equilibrium towards the inactive (R) state.

with the binding of agonists. As antihistamines may, at least theoretically, be either inverse agonists or neutral antagonists, it is clear that the term 'H₁-receptor antagonists' is erroneous. As a consequence, we suggest that the term 'H₁-antihistamines' be used as a generic descriptor of the drug class.

Because of their important implications for the proper understanding of drug action, the concepts of constitutive GPCR activity and inverse agonism are currently receiving considerable attention in the field of drug discovery. Furthermore, the observation that constitutive GPCR activity is often associated with mutant GPCRs has awakened interest in this phenomenon being the mechanistic basis for several genetic diseases.

The working model for GPCRs is a dynamic equilibrium between their inactive (R) and active (R*) conformations (Fig. 1a). Within this model, agonist-independent spontaneous isomerization of the receptors from the inactive to the active state would lead to a shift in the equilibrium towards apparent constitutive GPCR activity. Such isomerization involves conformational changes, which may occur either spontaneously in a wild-type GPCR or may be induced by specific mutations that disrupt intramolecular constraints within the GPCR [7,8]. An agonist will preferentially bind to the active state of the receptor to increase its stability and thus shift the equilibrium towards the active state (Fig. 1b), the degree of shift depending on whether the agent is a full or partial agonist (Fig. 2). In contrast, inverse agonists preferentially bind to the inactive state of the receptor and shift the equilibrium in the opposite direction, towards the inactive state (Fig. 1c), the degree of shift again depending on the nature of the inverse agonist (Fig. 2). Neutral

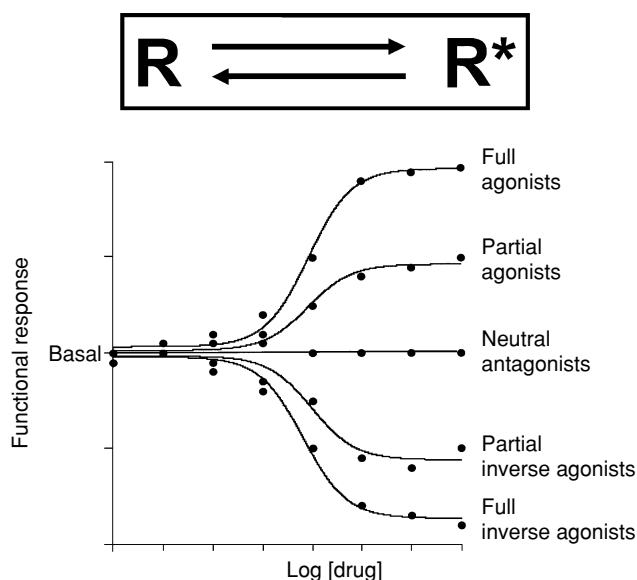


Fig. 2. On the basis of the two state model ligands may now be reclassified as agonists, both full and partial, which stabilize the active state (R*) and increase receptor signalling, inverse agonists, both full and partial, which stabilize the inactive state (R) and decrease basal receptor signalling, and neutral antagonists, which have equal affinity for both R* and R and therefore do not affect the equilibrium between the two states. Neutral antagonists will, however, reduce the ability of both agonists and inverse agonists to bind to the receptor.

antagonists, as stated above, do not discriminate between active and inactive states of the receptor and, consequently, bind to both, leaving unaltered the equilibrium between the two states (Fig. 2) but interfering with the subsequent binding of both agonists and inverse agonists.

Constitutive activity has now been shown for all four histamine receptors [9–12], including the human H_1 -receptor [5,13]. The identification of constitutive H_1 -receptor activity suggested that inverse agonism might be the mechanistic basis for the interaction of the so-called H_1 -antagonists with the H_1 -receptor. Transient expression of the human H_1 -receptor in COS-7 cells revealed constitutive activation of phospholipase C, resulting in a rise of the basal levels of InsP_3 (Fig. 3), indicating constitutive $G_{q/11}$ -coupling of the H_1 -receptor. Whereas mepyramine does not affect the InsP_3 accumulation in control cells, the increased basal levels of InsP_3 in H_1 -expressing COS-7 cells is potently inhibited. In contrast, neither H_2 nor H_3 antagonists affect this response. Furthermore, in accordance with the known H_1 -receptor stereospecificity, the enantiomers of cetirizine display stereospecific inhibition of constitutive H_1 -receptor activation. As expected, the potencies of H_1 -inverse agonists correlate well with their respective pK_i -values ($R^2 = 0.98$) [13].

Constitutive H_1 -receptor activity is not restricted to the activation of phospholipase C, but has also been shown for the stimulation of NF- κ B-mediated gene transcription. Using a NF- κ B reporter-gene assay, a large number of H_1 -antihistamines have been tested for their modulation of constitutive H_1 -receptor activity. Constitutive H_1 -receptor-mediated NF- κ B activation has been shown to be inhibited by all the clinically used H_1 -antihistamines tested to date, including cetirizine, ebastine, epinastine, fexofenadine, loratadine and mizolastine, indicating that all of these drugs are inverse H_1 -agonists [5]. None of these molecules have an intrinsic activity value close to zero, which would be suggestive of neutral antagonist activity. The negative intrinsic activities of the ligands vary between -1.00 for mepyramine and -0.72 for triprolidine. On the basis of these data, mepyramine should be reclassified as a full inverse agonist whereas various other tested H_1 -receptor antagonists show partial inverse agonism in the reporter-gene assay [5].

The notion of constitutive GPCR activity raises the question of the potential importance of this concept in physiological systems. It has been reported recently that H_3 -receptors in rodent brain show constitutive activity and that this is a mechanism for controlling histaminergic neuron activity *in vivo* [10,12]. At present no data on this issue are available for histamine H_1 -receptors. Interestingly, however, there are several pathophysiological conditions in which H_1 -receptor up-regulation has been reported. In the nasal mucosa of patients suffering from allergic rhinitis, H_1 -receptor mRNA is significantly up-regulated [14,15]. H_1 -receptor up-regulation has also been shown in vascular smooth muscle in atherosclerosis [14] and in specific brain areas upon seizures [16,17]. It is tempting to speculate that in these conditions the constitutive H_1 -receptor activity is also increased and may contribute to the disorder.

The discovery of inverse agonism has also raised the general question of potential differences in therapeutic outcome upon treatment with inverse agonists or neutral antagonists. An important issue that has been considered in this respect is the modulation of GPCR expression levels by inverse agonists. For example, it has been shown recently that, in contrast to the neutral antagonist burimamide, inverse agonists, like

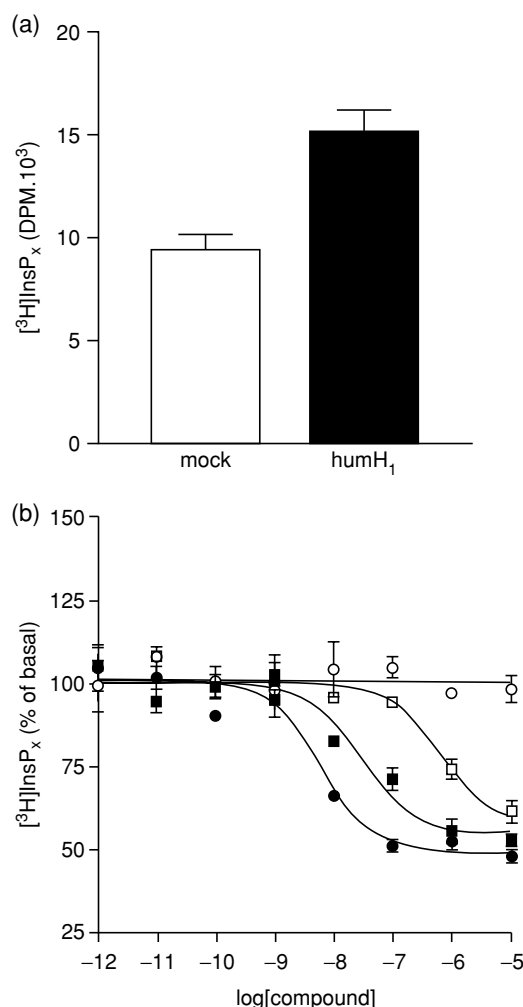


Fig. 3. (a) Effect of over-expression of the human H_1 receptor in COS-7 cells on the basal level of $[^3\text{H}]\text{inositolphosphates}$ ($[^3\text{H}]\text{InsP}$). (b) The effect of mepyramine on the basal $[^3\text{H}]\text{InsP}$ level in mock transfected COS-7 cells ($\circ$) or COS-7 cells expressing the human H_1 receptor ($\bullet$). To illustrate the H_1 -receptor pharmacology, the action of (R)- ($\square$) and (S)-cetirizine ($\blacksquare$) in H_1 -receptor-expressing cells are indicated as well. Data are reproduced from Bakker et al. ([13]) with permission from Elsevier Science BV.

cimetidine and ranitidine, cause an up-regulation of the H_2 -receptor expression [9]. Moreover, studies with the 5HT_{2C} -receptor [18] and the CB_2 cannabinoid receptor [19] have shown that besides GPCR expression, other cellular constituents, such as G-proteins, can be affected upon long-term exposure to inverse agonists. These data indicate clearly that differences in therapeutic outcome might be expected for inverse agonists and neutral antagonist at the H_1 -receptor. However, at present the identification of the first neutral antagonist for the H_1 -receptor is awaited. Thereafter, the therapeutic difference of the use if H_1 -inverse agonists and neutral H_1 -antagonists will need to be investigated.

The anti-inflammatory properties of H_1 -antihistamines

Ever since Arunlakshana and Schild [20] demonstrated in 1953 their ability to inhibit histamine release from mast cells,

numerous studies have been conducted, both *in vitro* and *in vivo*, to determine whether H₁-antihistamines possess properties, in addition to the inhibition of the effects of histamine, which would contribute to their clinical efficacy in the treatment of allergic disease. With the advancing knowledge about the molecular mechanisms underlying the effects of H₁-antihistamines, it is pertinent to reassess the possibility that the drugs may have anti-inflammatory properties and comment on their possible clinical relevance. In order to put these comments on a scientific basis, we have postulated that some anti-inflammatory effects of H₁-antihistamines are subsequent to their interaction with H₁-receptors while others are receptor-independent.

Receptor-independent anti-inflammatory effects

Inhibition of mast cell and basophil histamine release

While a specific H₁-antihistamine would theoretically prevent the actions of mast cell and basophil-derived histamine on its target organs, it would not prevent the actions of PGD₂, LTC₄, PAF or cytokines derived from these cells. Thus, to have a drug which prevents the generation of all mast cell and basophil mediator release rather than one which merely prevents the actions of histamine has obvious advantages. Early studies examining the effects of H₁-antihistamines on mediator secretion using guinea-pig and rat mast cells [21] showed clearly that H₁-antihistamines have the capacity to inhibit histamine release. This was confirmed in a later study [22,23] in which it was also established that there was no correlation between the concentrations of drugs which inhibited histamine release and their H₁-antihistaminic potency. Perhaps the most likely explanation of the mechanisms by which H₁-antihistamines exert these effects derives from the meticulous studies performed by Seeman and coworkers [24,25] in which they tested a series of lipophilic cationic drugs with local anaesthetic properties to advance the following mechanism of mast cell stabilization. They and others have suggested that the physical nature of these drugs, namely lipophilic molecules which carry a positive charge, allows them to associate with the cell membrane and competitively inhibit the binding of calcium thereby reducing the activity of calcium-dependent enzymes, such as calmodulin [24–27]. More recent studies, however, have suggested a direct inhibitory effect of H₁-antihistamines on calcium ion channels, reducing the inward Ca²⁺ current activated by intracellular Ca²⁺ store depletion [28–30].

Since the concentrations of drugs required to prevent mast cell and basophil histamine release *in vitro* are in the order of 1–10 µM, higher than those likely to occur *in vivo*, the question arises as to the clinical significance of this effect [31]. To test this, we administered two H₁-antihistamines cetirizine and loratadine, both at a dose of 10 mg orally, 4 h before the provocation of histamine release by intradermal injections of 3 mg/mL and 10 mg/mL codeine [32]. The results showed a clear inhibition of the ensuing weal and flare responses, demonstrating that the drugs had been absorbed and were bioactive. However, the use of dermal microdialysis to recover histamine release from the extracellular fluid, showed that neither drug reduced histamine release. Thus, it is unlikely that the inhibition of mast cell histamine release by H₁-antihistamines contributes to their beneficial therapeutic effects in the treatment of allergic and inflammatory reactions.

Inhibition of inflammatory cell activation The potential of H₁-antihistamines to inhibit indices of inflammatory cell activation has been studied widely [31]. These include the *de novo* generation, by membrane-associated enzymes, of pro-inflammatory products such as superoxide radicals (O₂[•]) and the arachidonic acid products, LTB₄ and LTC₄ and the release of granule associated products, such as neutrophil elastase and eosinophil cationic protein (ECP). Two possible unifying concepts have been suggested to be responsible for this diverse spectrum of effects, inhibition of calcium mobilization and the discharge of intracellular calcium stores [33,34] and inhibition of the activity of membrane-associated enzymes such as inhibition of protein kinase C [35] and NADPH oxidase [36,37]. However, as with inhibition of histamine release, the accumulated evidence indicates that there is no clear relationship between these effects of H₁-antihistamines and their H₁-antihistaminic potency [38,39]. While individual studies vary in detail from one another, probably because of differences in experimental conditions and provoking stimuli may influence the observed result, one feature common to all of them is that drug concentrations in excess of 10 µM are required for activity. This again suggests that the effects are likely to be histamine H₁-receptor-independent and have questionable clinical relevance [31].

Receptor-dependent anti-inflammatory effects

Effects of H₁-antihistamines on adhesion protein expression and the migration of inflammatory cells

The accumulation and activation of inflammatory cells, particularly eosinophils, at the sites of allergic reactions is a characteristic feature of allergic inflammation. The ability of an antihistamine to influence these events would obviously extend its therapeutic potential. The earliest clinical observations that an H₁-antihistamine could reduce allergen-induced eosinophil accumulation were obtained with cetirizine in the skin, using the skin window technique [40,41] in the nose [42] and in the airways [43].

A possible mechanism for the inhibitory effect of H₁-antihistamines on inflammatory cell accumulation and activation is their ability to down-regulate the activation of NF-κB, as described above [5]. NF-κB is an ubiquitous transcription factor which binds to promoter/enhancer regions of many genes regulating the production of a number of pro-inflammatory cytokines and adhesion proteins (Fig. 4). NF-κB may be activated by a variety of inflammatory mediators, including histamine [3–5] and TNF-α [44]. Low concentrations of the antihistamines cetirizine and azelastine have been shown to down-regulate NF-κB expression in parallel with inhibiting the generation of the cytokines, IL-1β, IL-6, IL-8, TNF-α and GM-CSF [45–47]. In clinical studies, several H₁-antihistamines, including, cetirizine [48], azelastine [49,50], loratadine [51] and levocabastine [52–54] have been reported to reduce conjunctival ICAM-1 expression. Also in the nose, cetirizine and loratadine have been reported to reduce the release of soluble ICAM-1 into nasal secretions in seasonal allergic rhinitis [55].

If these important anti-inflammatory effects are, in fact, secondary to their interaction with H₁-receptors, then they will occur with all clinically used H₁-antihistamines, the degree of effect being dependent on their H₁-antihistaminic potency and the dose at which the drugs are used.

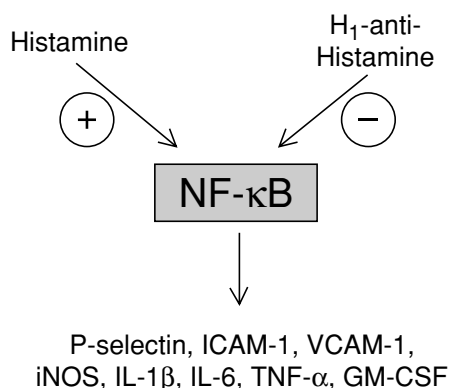


Fig. 4. Differential regulation of the activity of the transcription factor NF- κ B at constitutively active H₁-receptors by histamine and an H₁-antihistamine acting as agonist and inverse agonist, respectively. Via modulation of H₁-receptor activity, the activity of NF- κ B is attenuated in opposite directions by the two ligands. Active NF- κ B will migrate to the cell nucleus to affect the transcription and, consequently, production of many important mediators of inflammation.

Inhibition by H₁-antihistamines of the effects of bradykinin Bradykinin, a nonapeptide formed by the enzymatic actions of kallikrein on extracellular kininogen, plays a central role in the production of inflammation and pain. It is of particular interest in dermatology because of its ability to cause local oedema and increased blood flow in the skin and because activation of kinin pathways are believed to be involved in angioedema which occurs in some patients with urticaria, particularly those with hereditary angioedema and C1 esterase deficiency. When injected intradermally, bradykinin produces a weal and flare response which is, in appearance, very similar to that produced by histamine and histamine releasing agents. However, the possible involvement of histamine in the bradykinin-induced weal and flare response is controversial. H₁-antihistamines such as mepyramine [56], chlorpheniramine [57], terfenadine [58] and cetirizine [59,60] have all been reported to be inhibitory, suggesting that the response involves the release of histamine by bradykinin. However, assessment of extravascular histamine concentrations by dermal microdialysis showed that bradykinin released negligible quantities of histamine, certainly not sufficient to cause the observed weal and flare response [61,62]. These results are consistent with *in vitro* studies which report that human skin mast cells do not release histamine in response to bradykinin [63,64] and with the observation that the cutaneous sensation following the intradermal injection of bradykinin, a relatively long lasting 'burning' sensation, was quite different from that of histamine suggesting a different mechanism of action [61,62].

The ability of H₁-antihistamines to inhibit weal and flare responses to inflammatory mediators which induce small or negligible amounts of histamine is not unique to bradykinin, having been reported also for methacholine [40,65] and platelet activating factor (PAF) [66,67]. The mechanism(s) by which H₁-antihistamines abrogate these responses is far from clear. However, one hypothesis is interplay between their receptors, all of which are GPCRs. While it was originally thought that individual GPCRs acted as discrete units, there is increasing evidence that receptor oligomerization, and in particular dimerization, may play an important role in the molecular events leading to their activation [68,69]. Indeed, it has already been

reported that the bradykinin B₂-receptor has the capability of forming both homodimers and heterodimers which lead to changes in its responsiveness upon stimulation [70,71]. It is tempting to speculate therefore that heterodimerization of histamine H₁-receptors with B₂-receptors for bradykinin, M₃-receptors for methacholine and PAF-R for platelet activating factor may be responsible for the ability of H₁-antihistamines to inhibit the effects of all four stimuli. However, no direct experimental evidence is currently available on this issue.

The cardiovascular adverse effects of H₁-antihistamines

Although histamine itself has important cardiovascular effects and the cardioactive properties of 'classical' antihistamines have been known for decades, it is only in the last 15 years that the issue of a possible association between the use of H₁-antihistamines and cardiac arrhythmias has received considerable attention [72]. During this period, an increasing number of reports have shown an association between the consumption of astemizole or terfenadine, in particular, and the occurrence of prolongation of the QT interval, leading to the appearance of polymorphic ventricular arrhythmias, syncope, and even cardiac arrest [73,74]. After reviewing 25 case reports of associations between cardiac arrhythmia and the use of second-generation antihistamines, the FDA in 1990 introduced labelling changes for terfenadine, and in 1992 a prominent box warning was added. In addition, to increase the level of awareness of patients and physicians about the serious and potentially fatal cardiac adverse effects associated with the inappropriate use of terfenadine, 'Dear Health Care Professional' letters were sent to physicians and pharmacists in the US in 1990, 1992 and 1996. Following these letters, the number of cases reporting potentially fatal cardiac arrhythmias in association with terfenadine and astemizole has grown to over 200 [75]. Because of continuing problems, both terfenadine and astemizole were withdrawn from the market in several countries.

Predisposing factors to the cardiac adverse effects of H₁-antihistamines

Under standard therapeutic settings with conventional doses, terfenadine can be considered to be a pro-drug since it undergoes hepatic first-pass extraction and extensive metabolism into its carboxylate metabolite, called fexofenadine. In most normal subjects, the activity of the principle enzyme involved in this metabolism, CYP3A4, a member of the hepatic cytochrome P450 family, is sufficient to ensure that the levels of terfenadine in the plasma are below the detection limits. As the cardiac adverse effects are related directly to increased plasma levels of the unmetabolized parent drug, it follows that impairment of liver metabolism appears to be one of the main predisposing factor for the occurrence of terfenadine cardiotoxicity [75]. Indeed, most of the US cases reviewed initially by the FDA involved the concomitant use of inhibitors of CYP3A4, such as ketoconazole, itraconazole, and macrolide antibiotics, although patients with impaired liver function, due to conditions such as cirrhosis or ethanol abuse, were also realized to be at risk of developing arrhythmias. More recently, it has been recognized that other clinical conditions of pre-existing cardiac dysfunctions, such as congenital QT prolongation (see below), ischemic heart diseases, and congestive heart failure, or

electrolyte imbalance, such as hypokalemia and hypomagnesaemia, may also precipitate arrhythmic episodes induced by H_1 -antihistamines such as terfenadine and astemizole [72]. Finally, an additional risk factor which might precipitate adverse cardiac events upon exposure to H_1 -antihistamines is the simultaneous consumption of other QT-prolonging compounds such as tricyclic antidepressants and anti-psychotic drugs [76].

Ion channels involved in the control of cardiac repolarization: HERG1 K^+ channels as targets for H_1 -antihistamines

Most of the arrhythmic episodes precipitated by terfenadine or astemizole in predisposed patients were polymorphic ventricular tachycardias with 'torsade de pointes' ventricular fibrillation. 'Torsade de pointes' consists of a progressive modification of the amplitude and polarity of the QRS complexes on the surface ECG, which appear to be twisting around the isoelectric baseline, generally occurring in the setting of a marked bradycardia with prolongation of the QT interval (generally > 440 ms). In most experimental settings, the action potential prolongation which results in 'torsade de pointes' is consequent to the blockade of the repolarizing currents mainly carried by cardiac K^+ channels. Under physiological conditions, several classes of K^+ currents shape the action potential in cardiac cells [77]: the transient outward current (I_{to}), the delayed rectifier repolarizing current made up of both rapid (I_{Kr}) and slow (I_{Ks}) components, and the inward rectifier I_{K1} current, which participates in the final phases of repolarization. Other K^+ channels are also active during the action potential plateau in selective heart regions, or under specific pathological conditions. Such functional heterogeneity is matched by an extraordinary degree of structural heterogeneity, as revealed by the recent cloning of most of the genes encoding for cardiac K^+ channels [78].

A major contribution to the identification of the molecular mechanisms underlying the cardiac toxicity of antihistamines has been the study of the molecular genetics of the Long QT Syndrome (LQTS). This life-threatening genetically transmitted human disease is characterized by a marked prolongation of the QT interval on the ECG and by frequent episodes of 'torsade de pointes' ventricular arrhythmias and syncope or cardiac arrest usually occurring during conditions of psychological or physical stress [79]. Mutations in five genes have been associated to LQTS [80–84]. Two of these genes (HERG1 and K_vLQT1) encode for the main subunits of cardiac K^+ channels, one (SCN5A) for a Na^+ channel subunit, and the last two (MinK and MiRP1) for accessory subunits regulating K^+ channel function.

These studies provided a clear functional role for these genes encoding for cardiac ion channels. In particular, HERG1 (the acronym for Human Ether-a-Gogo-Related Gene – attributed to this gene as it was cloned by homology to another K^+ channel-encoding gene named Ether-a-Gogo [85]) was soon recognized to encode for the main subunit of the rapid component of the ventricular repolarizing current I_{Kr} [86]. The predicted protein encoded by HERG1 shares considerable sequence similarity with other voltage-dependent K^+ channels, displaying a putative topological arrangement with six α -helical transmembrane domains, the N- and C-termini located in the cytoplasm, a cyclic nucleotide-binding domain in the C-terminus, and a pore-forming region between the transmembrane segments S_5 and S_6 (Fig. 5). Mutations in HERG1 have been

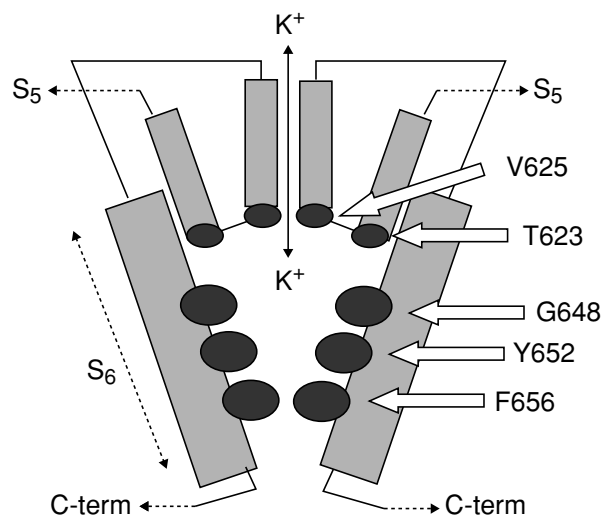


Fig. 5. Schematic drawing of the pore region and of the S_6 transmembrane segment of HERG1. Only two of the four channel-forming subunits of HERG1 channels are drawn. The residues T623 and V625 (in the P-region) and G648, Y652, and F656 (in S_6), which appear to be crucial for drug binding to HERG1 channels, are highlighted. In particular, terfenadine binding has been shown to be influenced by mutations at the Y652 and F656 aromatic residues. Modified from Vandenberg JI et al. ([103]).

proposed to impair the rapid component of the ventricular repolarizing current I_{Kr} , thus causing delayed repolarization, which represents a triggering mechanism for 'torsade de pointes'.

Soon after the description of HERG1 K^+ channels as the main constituents of the cardiac repolarizing current I_{Kr} , terfenadine [87] and astemizole [88] were shown to effectively block HERG1 K^+ channels with IC_{50} s in the nanomolar range of concentrations. On the basis of these results, it was suggested that cardiotoxic H_1 -antihistamines delay action potential repolarization by blocking HERG1 K^+ channels, thus predisposing the individual to the occurrence of an arrhythmic episode. The concentrations of terfenadine and astemizole required to produce blockade of HERG1 K^+ channels were within the range found in the clinical situations in which patients experienced cardiotoxic manifestations [75]. The predictive value of these *in vitro* studies has been confirmed by the results obtained with the main metabolic products of terfenadine and astemizole, namely fexofenadine and desmethyastemizole. Fexofenadine, which was shown to be ineffective in blocking HERG1 K^+ channels [87], appears to lack the cardiotoxic effects of terfenadine *in vivo* [89] even though this has been questioned recently [90]. Conversely, both metabolites of astemizole, desmethyastemizole and norastemizole, display potent inhibitory actions on HERG1 K^+ channels [91].

The important cardiotoxic effects exerted by terfenadine and astemizole, two of the most widely used of the newer non-sedating antihistamines, have prompted speculations on whether other molecules belonging to this therapeutic class might also have similar pharmacological effects [72]. However, a comparison of terfenadine, astemizole, loratadine and cetirizine using heterologous expression of HERG1 K^+ channels in *Xenopus* oocytes or HEK-293 cells and cells constitutively expressing HERG1 K^+ channels, showed these drugs

display marked differences [92]. Terfenadine and astemizole blocked $HERG1 K^+$ channels as predicted. Cetirizine, a piperazine derivative, was, however, shown to be devoid of $HERG1$ -blocking ability at concentrations of 1–30 μM , some 2–6 times higher than those occurring in the plasma of normal subjects after the administration of recommended daily therapeutic doses. This observation suggest that 'torsade de pointes' is not likely to occur during conventional therapy with this drug, providing support to the clinical observation that cetirizine does not display significant prolongation of the QT interval in experimental animals or humans [93].

Using the same expression systems, loratadine, a piperidinic antihistamine did not block $HERG1 K^+$ channels at concentrations up to 10 μM , but blockade was observed at higher concentrations [92,94]. Given that the concentrations of loratadine required to block $HERG1 K^+$ channels ($> 10 \mu M$) were at least 30 times higher than those achieved in the plasma during conventional therapy, these results might also explain the lack of cardiac side-effects associated with its use in humans and experimental animals. In fact, a recent pharmacosurveillance study showed that cetirizine and loratadine displayed the lowest incidence of heart rhythm disorders and cardiac deaths per million of defined daily doses sold among the most common non-sedating antihistamines [95]. However, the similarity between loratadine and terfenadine with respect to $HERG1$ channels blockade has been underlined by a recent study performed in isolated cardiac myocytes [96].

These techniques have recently also been applied to mizolastine, a new potent and selective H_1 -antihistamine available in Europe, which undergoes limited metabolic conversion by the hepatic CYPs 3A4 and 2A6. The results showed that mizolastine is able to block $HERG1 K^+$ channels to some degree [97]. Although caution should be exercised when extrapolating the possible *in vivo* consequences of the results generated from *in vitro* studies, and consideration taken of the relatively high concentrations of mizolastine required to inhibit $HERG1 K^+$ channels, these results may be of some clinical significance for those patients at risk of developing cardiac arrhythmias undergoing therapy with mizolastine.

Potential cardiotoxicity of older antihistamines

It has been reported recently that diphenhydramine, in concentrations below 10 μM , may block the repolarizing K^+ currents in guinea-pig ventricular myocytes and lengthen cardiac repolarization in humans [98]. Also, both diphenhydramine and hydroxyzine, the metabolic precursor of cetirizine, when taken in larger than therapeutic doses, have the ability of inducing QT prolongation, in some cases leading to the occurrence of cardiac arrhythmias. These observations prompted a study of the *in vitro* blockade of $HERG1 K^+$ channels by hydroxyzine and diphenhydramine [99]. The results suggest that both compounds, at similar concentrations to those found to block I_{K_r} in guinea-pig ventricular myocytes [98], exert an inhibitory action on $HERG1 K^+$ channels [99]. While higher than those expected following normal therapeutic doses of the drugs, these concentrations of hydroxyzine and diphenhydramine are similar to those found in the plasma in patients poisoned with these drugs. Interestingly the incidence of life-threatening ventricular arrhythmic events and cardiac arrests is higher in patients receiving 'over the counter' older antihistamines, particularly diphenhydramine, than in those receiving terfenadine or a

reference drug (ibuprofen) [100]. These experimental, clinical, and pharmaco-epidemiological data support the hypothesis that the older H_1 -antihistamines may be cardiotoxic in specific clinical settings, although the predisposing factors are difficult to unravel due little knowledge being available on the pharmaco-metabolic profile of such compounds.

Why $HERG1 K^+$ channels are major targets for drug-induced arrhythmias

Drug-induced QT prolongation and arrhythmias have been reported for a wide range of both cardiac and non-cardiac drugs. In addition to antihistamines, prokinetics (cisapride), psychotropics (antidepressants and anti-psychotics), antimicrobials (macrolides, fluoroquinolones, cotrimoxazole), anti-fungal agents (azole derivatives), and anti-marial agents (halofrantine), have been associated with such rare, but potentially fatal, adverse effect [76]. Despite having marked differences in their chemical structures, all of these drugs prolong action potential duration by inhibiting I_{K_r} . Therefore, the potential $HERG1$ -blocking ability is now regarded as a major issue for novel drug development. In recent months, two studies have attempted to clarify the structural basis for the high drug selectivity for $HERG1$ over other ion channels [101,102]. At least three different reasons have been suggested to account for the exquisite drug sensitivity of $HERG1 K^+$ channels [103]. First, $HERG1 K^+$ channels undergo fast inactivation and most of these drugs interact preferentially with the inactive state of the channel to stabilize it in the inactive conformation. Second, due to the absence in $HERG1 K^+$ channels of a Pro-X-Pro motif, which induces the formation of a kink in the α -helical structure in the pore-forming region of the S_6 domain of most other K^+ channels, the internal cavity of the pore in $HERG1 K^+$ channels is wider than in other K^+ channels. This allows the access to the internal part of the pore of molecules which would be otherwise excluded. Finally, the unusual presence of multiple aromatic rings lining the pore facilitates π -electron interactions between the channel and drugs which have aromatic rings in their chemical structure (Fig. 5). Structure-activity studies suggest in fact that most of the QT-prolonging drugs have at least one aromatic ring in their structure, which would be preferentially bound to these aromatic residues in the $HERG1$ pore.

Conclusions

Recent developments in molecular pharmacology and electrophysiology have allowed a deeper understanding of the mechanisms by which H_1 -antihistamines exert their effects. It is becoming clear that they interact with histamine H_1 -receptors as inverse agonists and not, as previously thought, as antagonists of the effects of histamine. This realization allowed the development of the concept of constitutive H_1 -receptor activity, i.e. the ability of the receptor to trigger downstream events even in the absence of ligand binding. While stimulation of the H_1 -receptor, a $G_{q/11}$ -coupled GPCR, classically activates inositol phospholipid signalling pathways resulting in $InsP_3$ and DAG formation and an increase in intracellular calcium, it has been shown recently to also activate the transcription factor, NF- κ B. This observation, together with the emerging knowledge of complex interactions between GPCRs, has allowed us to

postulate mechanisms for some of the anti-inflammatory effects of H₁-antihistamines. Finally, the finding that an interaction with HERG1 K⁺ channels is the mechanism by which H₁-antihistamines may cause potentially fatal cardiac arrhythmias, has allowed the development of preclinical tests, both *in vitro* and *in vivo*, to predict such activity, not only in antihistamines, but in all drugs used for systemic therapy.

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